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# Improving the endothelial dysfunction in type 2 diabetes with chromium and vitamin $D_3$ byreducing homocysteine and oxidative stress: A randomized placebo-controlled trial

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ABSTRACT

*Background:* Chromium picolinate (CrPic) and vitamin D3 are known as two antioxidant micronutrients. Through inducing endothelial dysfunction, oxidants such as homocysteine (Hct) and malondialdehyde (MDA) lead to cardiovascular disease in type 2 diabetes mellitus (T2DM). No published data has directly examined the effects of these two antioxidants on improving the endothelial dysfunction in T2DM throughreducing homocysteine and oxidative stress.

*Methods:* Subjects ($n = 92$) in this randomized, double blind, placebo-control study were randomly assigned to receive oral placebo (group I), $D_3$ (group II: 50,000 IU/ week), chromium picolinate (CrPic) (group III: 500 μg/ day), and both vitamin $D_3$ and CrPic (group IV) for four months. Fasting blood samples were drawn at study baseline and following intervention to determine Hct, MDA, total antioxidant capacity (TAC), total thiol groups (SHs), vascular cell adhesion molecule- 1 (VCAM-1), and plasminogen activator inhibitor-1 (PAI-1).

*Results:* After intervention, MDA significantly decreased in groups II and IV; TAC significantly increased in group IV, and SHs significantly augmented in group III; Hct was significantly reduced in groups II, III, and IV; and VCAM-1 significantly decreased in groups III and IV and PAI-1 was significantly reduced in groups II, III, and IV.

*Conclusion:* Our findings suggest that through reducing homocysteine and oxidative stress and improving endothelial dysfunction, chromium and vitamin $D_3$ co-supplementation might be predictive and preventive of cardiovascular diseasesassociated with T2DM.

IRCT, IRCT20190610043852N1, *registered 21 October 2019,* https://fa.irct.ir/user/trial/42293/view

## 1. Introduction

As the most serious manifestations of type 2 diabetes mellitus (T2DM), micor- and macrovascular complications such as atherosclerosis, nephropathy, and retinopathy are associated with long-term damage and failure of various organs [1]. Atherosclerosis is one of the main causes of mortality in T2DM. Over the recent years, many studies have concentrated on pathogenic mechanisms and their therapeutic interventions [2–4].

Endothelial dysfunction has recently been under special investigation as the primary cause of biochemical processes which ultimately lead to atherosclerosis. Oxidative stress (OS) and hyperhomocysteinemia

*Abbreviations:* T2DM, Type 2 diabetes mellitus; CrPic, Chromium picolinate; Hct, Homocysteine; MDA, Malondialdehyde; TAC, Total antioxidant capacity; SHs, Total thiol groups; VCAM-1, Vascular cell adhesion molecule- 1; PAI-1, Plasminogen activator inhibitor-1; Cr, Chromium; CβS, Cystathionine β-synthase; NF-κB, nuclear factor-κB transcription factor; TNF-α, Tumor necrosis factor-α.

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have prominent roles in the pathogenesis of endothelial dysfunction [5–7].

Certain components of the antioxidant defense system, including superoxide enzymes, glutathione peroxidase and catalase, acid ureic, bilirubin, and molecules containing the thiol groups are generated inside the body. However, other components such as vitamins C and E and beta-carotene are produced through diet [8,9]. Studies have shown that vitamin D supplementation and chromium (Cr) significantly reduce OS parameters with different mechanisms [10–13].

Other studies have investigated the combined antioxidant effects of chromium with vitamins C and E along with elements such as zinc; nonetheless, there are no studies on the antioxidant effects of the co-administration of chromium picolinate (CrPic) and vitamin D [14,15]. Moreover, to the best of our knowledge, there is no research on the effect of chromium or joint chromium and vitamin $D_3$ ($D_3$) on homocysteine (Hct), vascular cell adhesion molecule- 1 (VCAM-1), and plasminogen activator inhibitor-1 (PAI-1) in T2MD.

In the present study, we examined the effects of the co-administration of chromium picolinate (CrPic) and vitamin D on serum Hct, Malondialdehyde (MDA) as an indicator of lipid peroxidation, total thiol groups (SHs) as innate antioxidants, total antioxidant capacity (TAC) as a marker of antioxidant status, and VCAM-1 and PAI-1 as biomarkers of endothelial dysfunction.

## 2. Material and methods

### 2.1. Subjects

This randomized placebo-controlled trialwas conducted on additional samples saved from our previous study with the Ethical approval code IR.ARAKMU.REC.1395.466 and IRCT 2017052034038N1, patient willingness, and new research proposal with new codes of ethics and IRCT. To better correlate our previous and current study data, extra serum (stored at -80 °C) was used instead of re-sampling to measure other biochemical factors of the new proposal. The population consisted of 92 patients diagnosed with T2DM at least 5 years before the research. They were randomly divided into four groups, namely placebo (I), vitamin $D_3$ (II), CrPic (III), and both vitamin $D_3$ and CrPic (IV). We calculated the sample-size for serum homocysteine concentration as a key variable according to Aghamohammadi: $S_1 = 3.1$, $S_2 = 5.4$, $d = 4.2$, $\alpha = 0.05$ (type I error of 5%), $\beta = 0.2$ (type II error of 20%), power = 80%, and $K = 4$ [16]. A sample size of 19 individuals was reached for each group. However, considering the sample size of our previous study, 23 individuals were considered for each group.

Inclusion criteria were T2DM patients aged 35–70 years with the BMI ($kg/m^2$) of less than 35. T2DM was identified as having a FBS concentration greater than or equal to 126 mg/dl and HbA1c greater than 6.5٪, which is mentioned in the American Diabetes Association guidelines. During the trial, the subjects were instructed not to change their diabetes drugs or diet.

Exclusion criteria were (a) having congestive heart, renal and liver failure requiring pharmacological treatment, (b) being treated for insulin, hyperthyroidism, immunosuppressive, immunomodulating, and corticosteroids agents, subjects taking antihypertensive and lipid-lowering drugs were allowed to participate provided that dosages were stable for 6 months before enrollment, (c) smokers, (d) lactating and pregnant women, and (e) consumption of dietary supplements and/or weight-loss diets up to 6 months before enrollment in the study.

### 2.2. Ethical and safety considerations

This study was approved by the Ethics Committee on Human Research, Publication and Ethics at Arak University of Medical Sciences, Arak, Iran (IR.ARAKMU.REC.1398.131). Written informed consent was obtained regarding the use of the patients' additional serum samples to measure the biomarkers in this study. The trial was registered at the Iranian website (www.irct.ir) under IRCT20190610043852N1.

### 2.3. Study design and interventions

Study design and interventions were reported in our previous study [17]. In summary,

This study was conducted in the Diabetic Clinic of Imam Khomeini Hospital in Khomein, Iran, from August 2017 to January 2018. The study population consisted of 96 patients diagnosed with T2DM for at least five years before study entry, a stable dose of oral medications at least six months before screening, and without a significant change in their diet for more than six months before screening. They are randomly divided into four groups, including: (I) placebo of vitamin D3 at a dosage of one tablet/ week ($n = 23$), (II) vitamin D3 supplement at a dosage of 50,000 IU/ week ($n = 23$), (III) CrPic supplement at a dosage of 500 μg/ day ($n = 23$), and (IV) both vitamin D3 at a dosage of 50,000 IU/ week and CrPic at a dosage of 500 μg/day ($n = 23$) for four months, respectively.

### 2.4. Biochemical assessments

Blood samples of all the subjects were drawn after an overnight fast at the beginning of the study and after four months of supplementation. Aliquot samples of serums were saved following centrifugation (20 min; 3000 rpm) at −80 °C.

The serum levels of TAC, MDA, and total thiol groups were assayed using spectrophotometric method as previously described [18].

PAI-1 level was measured as an indicator of endothelial dysfunction by ELIZA kit (UK, abcam, ab108991). As another indicator of endothelial dysfunction, VCAM-1 level was measured by ELIZA kit (UK, abcam, ab223591).

Homocysteine levels were estimated by fully automated Chemi Luminescent Immune Assay method (model Immulite 2000 at the Laboratory of Clinical Analysis of Razi, Arak, Iran).

### 2.5. Statistical Analysis

Statistical tests were performed using SPSS version 17 (SPSS, Chicago, IL, USA). All data were expressed as means ± SD. The level of significance was set to $P < 0.05$. Normal distribution of the variables was examined by Kolmogorov-Smirnov Test. The groups were compared via the one-way ANOVA test, paired t-test, Wilcoxon signed-rank test, and Kruskal–Wallis one-way analysis of variance.

## 3. Results

This study was conducted on additional samples saved from our previous study conducted from August 2017 to January 2018. The patients' anthropometric and lipid, glucose, insulin resistance, and inflammatory patterns had been measured and reported in our previous article before and after the intervention [17].

Following the intervention, homocysteine was significantly reduced in groups II, III, and IV, and group I was significantly different from groups II, III, and IV (Table 1). Intra- day and inter – day values of homocysteine were below 2.5% and 3.9%, respectively. Thus, giving us confidence about of the reproducibility of the method. Also, Bias% for homocysteine was 1% which giving us confidence about of the trueness of the method (Table 2).

Regarding oxidative stress parameters, significant differences were observed in the mean of MDA before and after the intervention in all groups receiving the supplement. However, MDA did not significantly change in group I. There was no significant difference among the groups prior to the intervention whereas there was a significant difference between groups I and II and other groups and between groups III and IV (Table 1). Intra- day and inter – day values of MDA were below 5.3% and 8.3%, respectively. Thus, giving us confidence about of the

**Table 1**
The subjects' variables associated with the oxidative stress before and after the treatment in groups placebo, vitaminD$_3$, chromium picolinate and vitaminD$_3$ and chromium picolinate together.

| | placebo (I) ($n$=23) | | vitaminD$_3$ (II) ($n$=23) | | chromium picolinate (III) ($n$=23) | | vitaminD$_3$ and chromium picolinate (IV) ($n$=23) | | Statistical significance |
|---|---|---|---|---|---|---|---|---|---|
| MDA (μmol/L) | before 3.4±1.0<br>$P$< 0.193 | after 3.7±0.19 | before 3.7±0.70<br>$P$< 0.029* | after 3.25±0.62 | before 3.5±0.78<br>$P$< 0.01* | after 2.9±0.63 | before 3.6±0.90<br>$P$< 0.005* | after 2.9±0.4 | I×II: < 0.005<br>I×III: <0.05<br>I×IV: <0.05<br>II×III: <0.05<br>II×IV: <0.05<br>III×IV: NS |
| SHs (mmol/L) | 0.287± 0.133<br>$P$< 0.01* | 0.241± 0.07 | 0.259± 0.053<br>$P$< 0.188 | 0.359± 0.25 | 0.255 ± 0.09<br>$P$< 0.028* | 0.299 ± 0.06 | 0.275 ± 0.047<br>$P$< 0.094 | 0.320 ± 0.106 | I×II: <0.05<br>I×III: <0.05<br>I×IV: <0.05<br>others NS |
| TAC (μmol/L) | 962 ± 154<br>$P$< 0.447 | 934 ± 156 | 914 ± 208<br>$P$< 0.379 | 962 ± 162 | 883 ± 155<br>$P$< 0.166 | 931 ± 168 | 837 ± 108<br>$P$< 0.03* | 904 ± 83 | NS |
| Hct (μmol/L) | 18.09±2.84<br>$P$=.953 | 18.11±3.07 | 18.52±1.55<br>$P$=.000 | 15.60±2.77 | 18.26±2.34<br>$P$=.000 | 15.50±1.86 | 17.99±2.19<br>$P$=.000 | 14.49±2.82 | I×II: < 0.000<br>I×III: <0.000<br>I×IV: <0.000<br>II×III: NS<br>II×IV: NS<br>III×IV: NS |

Malondialdehyde (MDA); Sulfhydryl groups (SHs); Total antioxidant capacity (TAC);Homocysteine (Hct).
*P*- value: Differences in the percentage changes on mean value of initial reading between groups after the treatment and each group before and after the treatment (* significant, $P < 0.05$), Non- significant (NS).

**Table 2**
Assess the accuracy of the results Hct, MDA, TAC, and SHs.for intra-assay precision (Intra CV%) of 3 sample with low, middle and high-level of Hct, MDA, TAC, and SHs were tested for three replications in one day. Also, for inter-assay precision (Inter CV%) 3 sample with low, middle and high-level of Hct, MDA, TAC, and SHs were tested for 10 consecutive days.

| Assayed sample | Bias%* | concentration | Intra CV% | Inter CV% |
|---|---|---|---|---|
| **Hct** | **1** | 10.2 μmol/L | 2.4 | 3.83 |
| | | 15 μmol/L | 1.8 | 3.8 |
| | | 23.9 μmol/L | 1.6 | 3.3 |
| **MDA** | **3.2** | 1.7 μmol/L | 5.2 | 8.2 |
| | | 3.5 μmol/L | 3.3 | 8 |
| | | 5.9 μmol/L | 3 | 6.7 |
| **TAC** | **3.3** | 526 μmol/L | 3 | 3.4 |
| | | 883 μmol/L | 2 | 2.7 |
| | | 1267 μmol/L | 3 | 1.5 |
| **SHs** | **2** | 0.112 mmol/L | 2.55 | 5.4 |
| | | 0.46 mmol/L | 4.6 | 6.8 |
| | | 0.8 mmol/L | 4.3 | 5.5 |

Malondialdehyde (MDA); Sulfhydryl groups (SHs); Total antioxidant capacity (TAC);Homocysteine (Hct). *%Bias = [(test value – control value) / control value] * 100.

reproducibility of the method. Also, Bias% for MDA was 3.2% which giving us confidence about of the trueness of the method (Table 2).

SHs levels significantly decreased in group I, significantly augmented in group III, and nonsignificantly increased in groups II and IV. There was no significant difference among the groups before the intervention; however, groups I and II were significantly different from each other and other groups after the intervention (Table 1). Intra- day and inter – day values of SHs were below 4.7% and 6.9%, respectively. Thus, giving us confidence about of the reproducibility of the method. Also, Bias% for SHs was 2% which giving us confidence about of the trueness of the method (Table 2).

Following the intervention, TAC significantly increased in group IV and was not significantly different in groups I, II, and III (Table 1). Intra-day and inter – day values of SHs were below 3.1% and 3.5%, respectively. Thus, giving us confidence about of the reproducibility of the method. Also, Bias% for SHs was 3.33% which giving us confidence about of the trueness of the method (Table 2).

VCAM-1 was significantly reduced in groups III and IV after the intervention, but it did not change significantly in groups I and II. Groups I and II were significantly different from groups III and IV following the intervention (Table3).

After the intervention, PAI-1 decreased significantly in groups II, III, and IV, and there was a significant difference between all groups (Table3).

## 4. Discussion

In subjects with T2DM, one of the main causes of micro- and macrovascular complications is vulnerability to oxidative damage [1]. Oxidative damage and hyperhomocystenemia induce endothelial dysfunction as the primary cause of biochemical processes ultimately leading to atherosclerosis. Studies have shown that in these individuals, oxidative stress and hyperhomocystenemia are induced due to the lower status of micronutrients such as trace elements and vitamins [19–21]. Deficiency in Cr and vitamin D has been introduced as an aggravating factor in the progression of T2DM [14,22,23].

In the current study,vitamin D significantly reduced homocysteine. The metabolism ofhomocysteine, as an independent cardiovascular risk factor, is done by remethylation to methionine (to help folate and vitamin $B_{12}$) and the transsulfuration to cystathionine in the presence of vitamin $B_6$ and cystathionine β-synthase (CβS) [24]. The mechanism through which vitamin D reduces serum homocysteine is yet to be elucidated. Kriebitzsch showed that vitamin D increased the expression of CβS in MC3T3-E1 cells [25]. Pham et al. 2016 reported that serum homocysteine concentrations were reduced by the improvement in vitamin D status [26]. Mao et al. (2016) observed vitamin D to be positively correlated with folic acid and B12 [27]. Amer et al. (2014) investigated people with a vitamin D concentration of 21 ng/mL or less. They found an inverse relationship between vitamin D concentration and homocysteine levels, but the mechanism has not been investigated [28].

Furthermore, in the current study, CrPic significantly reduced homocysteine. This is the first study to investigate the effects of chromium on homocysteine levels, but the exact mechanism of this relationship is still unknown. Due to the reducing effect of CrPic on oxidative stress in this study, one possible mechanism might be increased CBS activity due

**Table 3**
The subjects' variables associated with endothelial dysfunction before and after the treatment in groups placebo, vitamin$D_3$, chromium picolinate and vitamin$D_3$ and chromium picolinate together.

| | placebo (I) ($n = 23$) | | vitamin$D_3$ (II) ($n = 23$) | | chromium picolinate (III) ($n = 23$) | | vitamin$D_3$ and chromium picolinate (IV) ($n = 23$) | | Statistical significance |
|---|---|---|---|---|---|---|---|---|---|
| | before | after | before | after | before | after | before | after | |
| VCAM-1 (μmol/L) | 1.38 ± 0.78 | 1.41 ± 0.78 | 1.307 ± 0.5 | 1.26 ± 0.59 | 2.92 ± 0.75 | 1.37 ± 0.53 | 2.71 ± 0.68 | 1.24 ± 0.46 | I × II: <NS<br>I × III: <0.05<br>I × IV: <0.05<br>II × III: <0.05<br>II × IV: <0.05<br>III × IV: NS |
| | $P < 0.808$ | | $P < 0.715$ | | $P < 0.0001$* | | $P < 0.0001$* | | |
| PAI -1 (μmol/L) | 15.21 ± 4.78 | 14.36 ± 2.7 | 14.97 ± 2.58 | 13.39 ± 2.94 | 17.79 ± 2.94 | 14.83 ± 2.39 | 17.45 ± 3.63 | 13.9 ± 1.59 | I × II: < 0.05<br>I × III: <0.05<br>I × IV: <0.05<br>II × III: <0.05<br>II × IV: <0.05<br>III × IV: <0.05 |
| | $P < 0.616$ | | $P < 0.033$* | | $P < 0.004$* | | $P < 0.001$* | | |

Vascular cell adhesion molecule 1 (VCAM-1); plasminogen activator inhibitor-1 (PAI-1).
*P*- value: Differences in the percentage changes on mean value of initial reading between groups after the treatment and each group before and after the treatment (* significant, $P < 0.05$), Non- significant (NS).

to oxidative stress reduced by CrPic. Niu et al. (2018) reported that the activity of reduced CBS was ~2- to 3-fold higher than that of the oxidized enzyme [29]. Finally, the combined use of CrPic and vitamin D supplementations significantly reduced homocysteine. The limitation of our study on the effects of vitamin D and chromium on homocysteine was that vitamin B12, B6, and folic acid levels were not measured. Future research is to examine the relationship between chromium and vitamin D both separately and together with folic acid, B12, and B6 levels and their impact on the expression and activity of enzymes (such as CβS) involved in the homocysteine metabolism pathway.

In this study, vitamin D significantly reduced MDA, but the increase in TAC and SHs was nonsignificant. Most studies have reported that oral vitamin D supplementation reduces MDA and TAC in T2DM [23,30–32]. However, findings are inconsistent and some studies have not revealed positive effects [33,34,13]. contradiction might be attributed to the differences in dose, route, and duration of vitamin D supplementation.

Vitamin D is able to reduce OS through upregulating cellular glutathione and antioxidant systems such as glutathione peroxidase and superoxide dismutase [10].

Higher levels of systemic inflammation, glycoxidation, and lipoxidation biomarkers are the consequences of vitamin D deficiency [35]. Shab-Bidar et al. observed vitamin D receptor (*Bsm*I) genotypes to influence inflammatory and oxidative stress responses with altered vitamin D intake in subjects with T2DM [35]. Vitamin D supplementation can ameliorate OS so as to improve insulin resistance and reduce blood pressure [35]. By reducing the activation of the pro-inflammatory transcription factor nuclear factor-κB, the vitamin D receptor plays an intrinsic inhibitory role in inflammation [35]. Oxidative stress and inflammation are closely related pathophysiological processes, with one easily induced by the other [36–38]. Accordingly, both processes are simultaneously found in many pathological conditions. We revealed that vitamin D reduced tumor necrosis factor-α (TNFα) and insulin resistance in T2DM [17].

In the current study, CrPic significantly reduced MDA and increased SHs, but the increase in TAC was nonsignificant. Lai found that Cr (1000 μg as Cr yeast) reduced TAC in T2DM [39]. According to our results and the findings of other studies, chromium can be introduced as an antioxidant [17,40–42]. Cheng et al. observed that Cr reduced MDA while augmenting TAC in T2DM [41]. Uslu et al. showed that CrPic significantly reduced the MDA levels in the heart and liver tissues in T2DM rats [43]. The exact mechanism of chromium antioxidant activity is yet to be known. Given the role of insulin perturbations in the formation of lipid peroxidation products such as MDA, it can be concluded that a possible mechanism for the decreasing effect of chromium on MDA is the reduced serum insulin level or its improved action by chromium [14]. Furthermore, chromium reduces MDA via depressing the formation of trichloromethyl radicals ($CCl_3$) from carbon tetrachloride ($CCl_4$) as the inductor of lipid peroxidation and reducing the age-related accumulation of lipofuscin [14,44]. Tezuka et al. reported that Cr reduced the lipid peroxidation in mice through inhibiting $CCl_4$ production [44].

Ultimately, the combined use of CrPic and vitamin D supplementations significantly reduced MDA and increased TAC, but the increase in SHs was nonsignificant. The nonsignificant changes in the SHs of group IV, TAC of group III, and TAC and SHs of group II might be ascribed to the relatively small number of subjects in each group ($n = 23$) which might cause subtle differences. More refined study designs with larger sample sizes are recommended.

In the current research, vitamin D did not significantly reduce VCAM-1, but PAI-1 decreased in group (II). Campos et al. revealed that vitamin D improved endothelial dysfunction by reducing VCAM-1 and PAI-1 in obese adolescents [38]. Naeini et al. reported that vitamin D reduced the serum levels of VCAM-1 in end-stage renal disease patients [45]. Nevertheless, Talasaz et al. did not observed any significant differences in the markers of endothelial function such as VCAM-1 in T2DM patients receiving vitamin D [46]. Given the decreasing effect of vitamin D on oxidative stress and TNF-α in these patients, and waiting for the activation of nuclear factor-κB transcription factor (NF-κB), this supplement was expected to reduce VCAM-1 in the current study. No significant reduction in the amount of VCAM-1 in this study might be due to the relatively small number of participants, hence the need for more refined study designs with larger sample sizes.

PAI-1 is involved in fibrinolysis and thrombogenicity. Increased PAI-1 expression accelerates the development of thrombosis, atherosclerosis, and vascular injury. Mansouritorghabe et al. (2013) reported that the serum level of vitamin D had no effects on PAI-1 in diabetic patients [47]. Wu-Wong et al. (2007) showed that vitamin D receptor (VDR) had a reducing effect on the expression of PAI-1 in coronary artery and aortic smooth muscle cells [48]. However, Petrie et al. (2004) observed vitamin D to stimulate the expression of PAI-1 in human umbilical vein endothelial cells [49]. These inconsistent results are possibly due to the difference between VDR expression and its response to vitamin D in different cells. In their clinical trial, Amarasekera et al. (2017) found that vitamin D supplementation for 12 weeks did not influence PAI-1 in healthy volunteers with 25(OH)D levels <75 nmol/L [50]. However, the contradiction in the results of clinical trial studies may be due to the differences in dosage and duration of treatment. Another possible mechanism for the reduced expression of this molecule by vitamin D is the reduction in homocysteine by this vitamin. Studies have shown the positive effect of homocysteine on the expression of PAI-1 [51].

Finally, Alyami et al. surveyed the randomized control trial articles

(2009–2014) on the effects of vitamin D supplementation on endothelial function. They reported that RCTs articles did not confirm the beneficial effect of vitamin D on vascular reactivity [52]. Intervention studies with more detailed designs (duration, dose, sample size, control conditions of the patients during treatment and the use of double-blind and placebo-controlled crossover models) are required to more precisely specify the regulatory role of vitamin D in endothelial function.

In this study, CrPic significantly reduced VCAM-1 and PAI-1 possibly because it reduced oxidative stress and TNF-α. VCAM-1 was expressed on the endothelial cells during inflammatory diseases via several mediators, including ROSs and TNF-α [53,54]. ROSs and TNF-α are involved in the activation of NF-κB [55], which induces the expression of VCAM-1 under inflammatory conditions. CrPic was shown to reduce ROSs and TNF-α in T2DM [17]. Therefore, CrPic might inhibit NF-κB through reducing inflammation and oxidative stress. Given the reduction of homocysteine in these subjects and the previous studies on the effect of homocysteine on NF-κB activation, a possible mechanism for the effect of this micronutrient on the expression of VCAM-1 and PAI-1 seems to the reduced homocysteine and consequently the decreased activity of NF-κB [56]. A detailed survey on the molecular mechanism of the effects of chromium on VCAM-1 and PAI-1 expression is suggested in future studies.

In the current research, combined use of CrPic and vitamin D significantly reduced VCAM-1 and PAI-1. It is proposed that the effects of these supplements on nitric oxide, antioxidant enzymes, and effective molecules in the expression of VCAM-1 and PAI-1 such as NF-κB be investigated to better fathom the exact mechanism of chromium and vitamin D.

## 5. Conclusion

In summary, these findings suggest that CrPic and vitamin D are effective in minimizing homocysteineand oxidative stress and improving the endothelial dysfunction in T2DM. Further research with more detailed design is recommended.

## Author statement

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## Consent for publication

Not applicable.

## Declaration of Competing Interest

None to declare.

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